

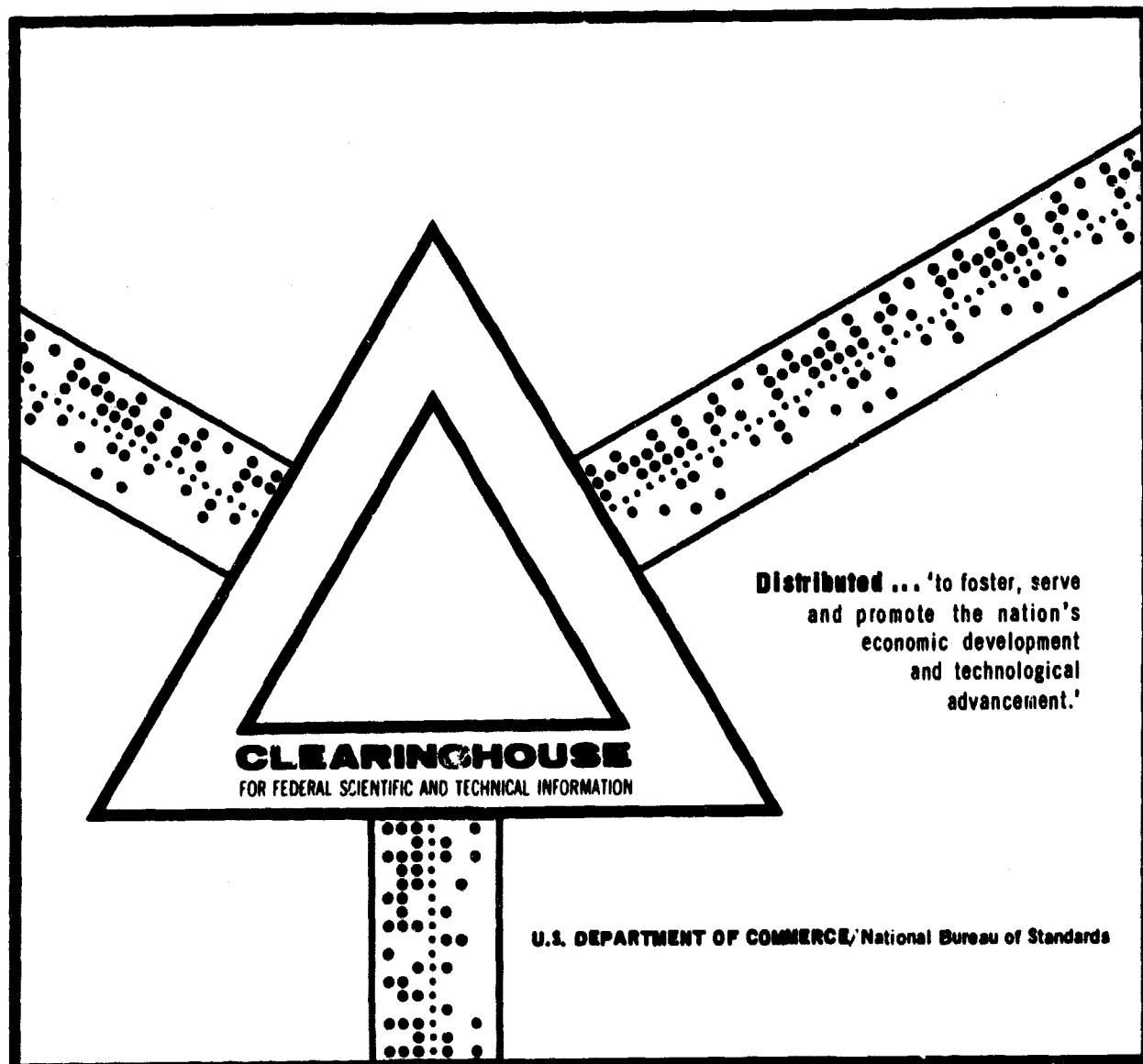
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CALCIUM, MAGNESIUM AND PHOSPHORUS METABOLISM
DURING PROLONGED EXPOSURE TO CARBON DIOXIDE

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Royal Naval Personnel Research Committee
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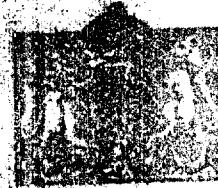


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PROLONGED EXPOSURE TO CARBON DIOXIDE

by

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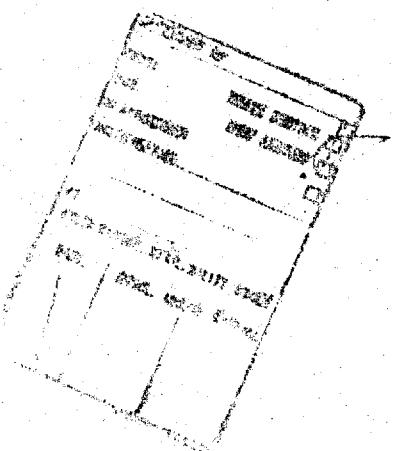
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SUMMARY

The daily urinary excretion of calcium, magnesium, and phosphorus was determined on 12 healthy volunteers during a period of 22 days exposure to an atmosphere containing 1% CO₂. Serum calcium fell significantly during the period, whilst the urinary excretion of calcium fell during the first week, and then rose again subsequently. The serum magnesium remained remarkably constant, and the urinary magnesium exhibited a very similar initial pattern to calcium but did not rise again to the same extent. The serum inorganic phosphate did not change significantly but the urinary output of phosphorus fell progressively over the whole period of exposure. These results are compatible with the view that acclimatization to CO₂ may result in altered renal handling of some of the substances involved in calcification of tissues. Such hypercalcification has been recorded in animals exposed to a similar environment, and it is suggested that the altered Ca and Mg ratio may be a factor in this hypercalcification.

INTRODUCTION

The present study is part of a wider investigation into the physiological effects of environment on submarine patrols. It is known that experimental animals exposed to 1.5% CO₂ for long periods develop calcification of the kidney (Schaefer and Hiemoller, 1960) and it has been argued that this condition may apply to personnel subjected to increased CO₂ tension for prolonged periods in submarines. The aetiological factors involved in calcification and renal stone formation are many and diverse, such as renal tubular acidosis, hyperparathyroidism, or anatomical abnormalities in the urinary tract. Albright et al. (1953) suggested that a high urinary calcium was a factor in renal stone formation, but Hodgkinson et al. (1958) found many symptomless hypercalciuric individuals in a normal population. Other urinary constituents have been implicated by some workers. Modlin (1967) suggested that a high Na/Ca ratio protected against calcium precipitation, but Papadimitriou et al. (1968) examined the urinary electrolytes in a group of stone formers and a group of controls, and concluded that there were no significant differences in the Na/Ca ratio. Evans et al. (1967) found magnesium to be significantly lowered in some of their normocalciuric stone formers. The role of magnesium was felt by Oreopoulos et al. (1968), to be important in the process of stone formation, particularly if the ratio Mg/Ca was low. Isaacson (1968) from his study concluded that there was no evidence for unique abnormalities in Na or Mg in nephrolithiasis and that although hypercalciuria relative to Na, K, Mg osmolality and ionic strength was a feature of stone formers, the most consistent finding was hypercalciuria relative to osmolality.

The formation of calcium salts in an insoluble state is evidently a multi-factorial process, and the investigation of a single aetiological factor is unlikely to be productive. In our own series however, (unpublished), stone formers did exhibit some very low urinary magnesium values which were well below the commonly accepted values. In this study a group of healthy individuals were examined to see if any metabolic effects arose from breathing 1% CO₂ for a period of three weeks which might alter the renal output of Ca, Mg and P, those being some of the factors which may be relevant to renal stone formation.

MATERIALS AND METHODS

Volunteers for this study were twelve healthy members of a submarine crew. Their ages ranged between 20 and 35 years, and none had any history of renal or skeletal pathology. All were subjected to an atmosphere of normal air containing 1% CO₂. The composition of the atmosphere was monitored 4 hourly with an installed infra-red atmosphere analyser, a minimum of three readings were taken on each occasion, from different parts of the vessel. Diet was free both before and during the experimental period, the only difference being in the use of distilled water instead of tap water, and the use of a milk substitute for beverages. The tap water normally taken had a Ca concentration of only 30 p.p.m., and the milk substitute differed only slightly from milk. Calcium and magnesium intake was thus similar before and during the experiment.

The period of exposure to this environment was twenty-two days with brief periods of surfacing when the mean concentration of CO₂ fell to not less than 0.5%. Fasting blood for calcium, magnesium, phosphorus, and creatinine, were collected on the second, ninth, and seventeenth days of the experiment. Aliquots of the first morning specimen of urine were collected on the day before and every day of the investigation. All sera and urine were frozen at -20°C within minutes of collection.

Analyses for calcium and magnesium were made on a Pye-Unicam SP90 atomic absorption spectrophotometer using the method supplied by the manufacturer. Creatinine and phosphate analyses were carried out on the Auto Analyser (Technicon) also using standard methods. The concentrations of Ca, Mg, and P were expressed as mg/100 ml of glomerular filtrate (G.F.) using the equation, (e.g. calcium).

$$\text{Calcium excretion, } \text{Ca}_E = \frac{\text{U}_{\text{Ca}} \times \text{P}_{\text{Cr}}}{\text{U}_{\text{Cr}}}$$

Where U_{Ca} = urinary calcium in mg/100 ml.

P_{Cr} = Plasma creatinine in mg/100 ml.

(Peacock and Nordin, 1968)

Statistical methods used were those from Documenta Geigy, Scientific Tables (6th Edit)

RESULTS

The atmospheric CO_2 level throughout the period of study was 1.02 (S.D.0.19) except for three brief periods when the CO_2 concentration fell to a lowest mean value of 0.53%.

The daily mean urinary excretions of calcium, magnesium and phosphorus are shown plotted in Figs. 1a, b and c as mg/100 ml G.F., versus the time of exposure to CO_2 . The calculated ratio of Ca/Mg for each day is shown in Fig. 1d.

The mean values of the 12 subjects for serum calcium, magnesium and phosphorus are shown in the following Table.

TABLE I

Serum	Ca	Mg	P
day 2	10.2	1.99	3.43
day 9	9.9	2.00	3.48
day 17	9.7	1.99	3.46

The relationships between the serum and urine levels of calcium and magnesium are illustrated in Figs. 2a and b.

It can be seen from Fig. 1a, that Ca_E rises steeply up to the second day, and then falls sharply to the fifth day. The output is then cyclical in pattern up to and above the initial level. Although these fluctuations are marked the net change in mean values is small and negative, the cumulative sum of the differences from the initial value before exposure, being nearly zero.

A similar pattern is shown by magnesium Fig. 1a, the correlation between those two being significant ($P < 0.01$), but it is evident from Fig. 1b, that Mg_E tends to fall over the period of exposure. Phosphorus shows the same initial pattern as the other two elements, up to the eighth day, but after this no real similarity in behaviour is noticeable. No significant correlation was demonstrable between P_E and Mg_E , Ca_E . Phosphate excretion is diminished to a greater extent than the other two, and the cyclic pattern was unrelated to the fluctuations in CO_2 concentration, as indeed were both Mg_E and Ca_E .

The ratio of Ca/Mg is seen to rise (Fig. 1d), this rise being largely due to lowered Mg_E rather than raised Ca_E .

If the whole mean output of Calcium (Ca_E) from a given individual for the twenty-two days of test is plotted versus the serum values from the latter part of the investigation, then the scatter graph in fig. 2a results. A distinct trend is seen but it is just not significant ($P > 0.05$). However, when Ca/Mg_E is plotted versus the serum calcium values (Fig. 2b), a much better relationship is seen which is highly significant ($P < 0.01$). The mean serum calcium is seen to fall, (table 1) and this fall is significant ($P < 0.05$). The serum inorganic phosphate and magnesium do not show any change in mean value.

DISCUSSION

The major environmental difference experienced in this study was the relatively high level of carbon dioxide compared to normal air. Other factors which may be pertinent, are dietary and occupational. Diet was free and did not measurably alter ashore and afloat, so the mineral intake presumably did not change to any significant extent. Similarly the work level did not undergo any significant change, the two levels of activity before and during the trial being classified as sedentary. A change which has been recorded in similar circumstances by Schaefer et al, (1963) is a diminution of urinary volume, and this has been confirmed by our own unpublished observations. The fall is of the order of some 20% and this may play a significant role in the kinetics of mineral handling by the kidney.

Acclimatization to CO_2 and its effect on calcium and phosphorus metabolism has been studied by Schaefer et al. (1963), who showed that there were two phases, one of uncompensated respiratory acidosis lasting up to 23 days, followed by a period of compensated respiratory acidosis. The metabolic effects of breathing 1.5% CO_2 in their study lasted for several weeks after entering normal air, and they concluded that their results were compatible with a storage of calcium and CO_2 in bone, as a release of both of these substances were seen on de-acclimatization. The results of that study would imply that the changes seen were not explicable by increased faecal excretion or diminished absorption, but these factors cannot be ruled out.

The initial rise in calcium excretion seen within two days of entry to the raised CO_2 level was reflected in the magnesium and phosphorus curves (fig. 1). This would seem to indicate a rapid physiological response to breathing air of this composition, and seems to imply also that other effects such as diet, degree of hydration, would not operate at such an early stage. The next stage up to the fifth day shows a marked downward trend for calcium, again reflected by the other two ions. The common renal tubular handling of calcium and magnesium is widely accepted (Hanna et al. (1961)) and the similarity in the two excretion patterns of calcium and magnesium is seen in Fig. 1a and 1b,

to be a significant one. However, the similarity is not complete, and the rise to the initial level of calcium concentration is not seen for magnesium, which is relatively lowered compared to calcium. Consequently the ratio $\text{Ca}_{\text{E}}/\text{Mg}_{\text{E}}$ goes up rather more due to diminution in magnesium output, than to increase in calcium excretion. It is of interest to compare the output of urinary calcium with the serum levels for calcium over the period of study (Fig. 2a). These blood levels were taken fasting, and it is usual to find that a fasting calcium level is stable on a moderately stable dietary intake, so that the fall shown is a real one, and confirms the findings of Schaefer et al (1963). This fall in serum calcium (Table 1) is still apparent at a time when the urinary calcium excretion is increasing. The filtered load for calcium is the product of the amount of ionised calcium in the plasma and the volume filtered. If the serum calcium falls, the filtered load should decrease unless the volume filtered at the glomerulus is increased. Under the conditions of this experiment a reduced rather than increased urinary volume would be expected.

The correlation seen in Fig. 2a is not high, probably because of the complexity of calcium homeostasis, but some dependence is seen, although it is just not significant at the 5% level. It is possible that the first phase, up to the sixth day, represents a storage of calcium which is then released and then excreted subsequently by the kidney, without a rise in the plasma level.

The results for Mg_{E} (Fig. 1b) seem to represent a diminution of urinary output without any change in the serum level. The homeostatic mechanism for magnesium involves the considerable flexibility of the nephron to excrete magnesium, and keep the serum level constant (Steele et al. 1968). The magnesium in the serum of these subjects remains remarkably constant. Not merely the mean values, but the individual results fluctuated by a figure well within the experimental error of duplication. If this constant level is associated with a diminished output of this ion, then some storage is possible.

It is of interest to see that magnesium output is affected so readily by the environmental stress applied in this situation. Magnesium as a largely intracellular ion is not regarded as a labile physiological entity. Phosphorus on the other hand (Fig. 1c) is dependent to some extent on diet, and is a fairly labile species. It is likely that the diminution in phosphate recorded here is a highly significant response to the requirements of the body to neutralise the CO_2 breathed in. The ability of phosphate to be excreted in several acid forms make it a very useful anion in the regulation of acid elimination. However, Schaefer (1963) showed that the regulatory role was anomalous, and the behaviour of phosphate in this study is not explicable in the absence of knowledge of other buffer changes in the body. The plasma inorganic phosphate mean values shown in table 1 show no significant overall change, but the individual levels showed considerable fluctuations.

The ratio of calcium to magnesium excretion ($\text{Ca}_{\text{E}}/\text{Mg}_{\text{E}}$) shows a cumulative rise over the period of exposure, largely due to fall in magnesium. Magnesium may well be important in the solubility of calcium salts, and it has been reported by Ward (1964), that nephrocalcinosis occurs in Mg depletion. Such effects on solubility could be a function of the formation constants of complexes of calcium and magnesium with anions such as oxalate, citrate and phosphate. The altered ratios of calcium to magnesium may well predispose to seed formation in the urinary tract. Such a nucleus is difficult to redissolve, and growth is more likely than dissolution, (Lonsdale, 1968). The influence of magnesium concentration is illustrated in Fig. 2b where the $\text{Ca}_{\text{E}}/\text{Mg}_{\text{E}}$ ratio correlates to a much more significant extent than does Ca_{E} alone, indicating perhaps that parathyroid function is more concerned with the two ions together than either alone.

That carbonate is an integral component of the bone crystal complex in man and other species, was shown by Pellegrino and Blitz (1968). Bichanan and Nakao (1952) showed that in rats 30% of the bone carbonate was exchanged in 12 days, while the rest appeared to be largely non-exchangeable. The possibility that the storage of CO₂ in the skeleton does occur has been strengthened by the observations of Lemann et al. (1964), that fixed buffers, probably skeletal, are called into play during experimental chronic acidosis in man. The role of bone as a buffer is not so important in the defence of pH, as the bicarbonate system in the tissues, but it seems likely that in chronic respiratory acidosis, some of the CO₂ could be initially stored in bone, to be released when the acidosis is relieved. Ca and Mg may be involved in the storage, and the results obtained here may be compatible with this idea.

Whether conditions of mild metabolic stress such as those encountered in this investigation, have a cumulative effect on idiopathic hypercalcification and renal stone formation is speculative, but certainly growth of renal stones has been observed to occur over as short a time as 24 hours (Gasser and Preisinger, 1958), so that such stress may have a contributory effect and provide the conditions for calcium salt deposition.

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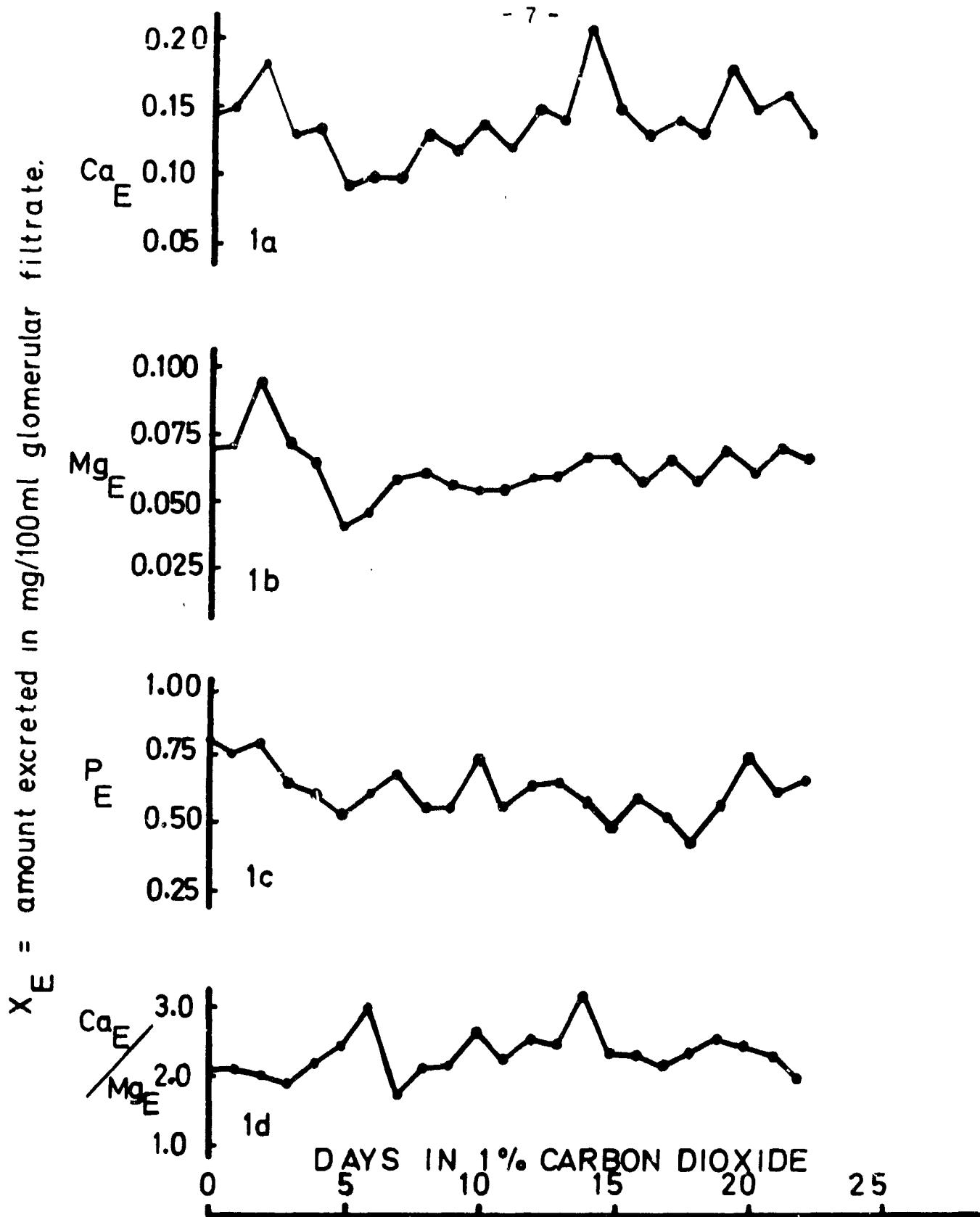


FIG. 1. Mean urinary excretion of calcium, magnesium and phosphorus. (Each day's value is the mean of 12 subjects).

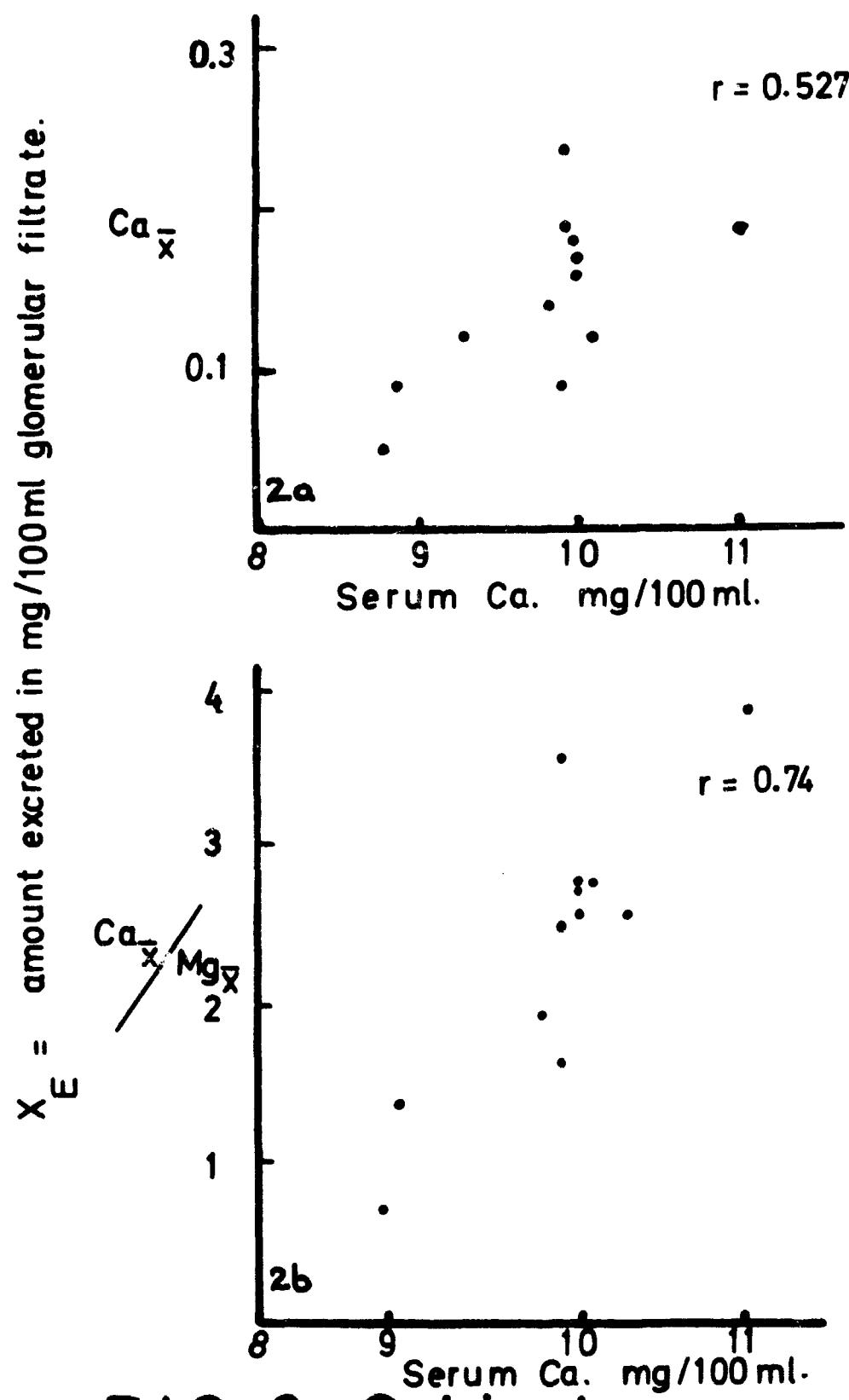


FIG. 2. Subject mean values versus serum calcium.